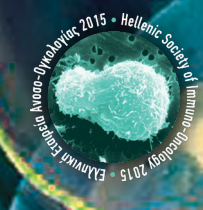
Hellenic Society of Immuno-Oncology



9th Symposium on Advances in Cancer Immunology and Immunotherapy

30/11-02/12, 2023

Royal Olympic Hotel

ATHENS

SCIENTIFIC PROGRAM

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Welcome Letter

Dear Friends and Colleagues,

It is a great pleasure to welcome you to the "9th Symposium "Advances in Cancer Immunology & Immunotherapy" in the city of Athens, Greece.

The 9th Symposium "Advances in Cancer Immunology & Immunotherapy" is an intensive, three days interactive meeting during which invited speakers will address the current and accumulated knowledge of basic immuno-biology and translational cancer research, as well as therapeutic developments in the field of cancer immunotherapy according to the newest clinical trials.

The scientific objectives of the 9th Symposium "Advances in Cancer Immunology and Immunotherapy" are focused on the: (a) predictive and prognostic biomarkers for immunotherapy (b) mechanisms of action of different immunotherapeutic treatments (c) the efficacy and safety data of cancer immunotherapy regimens; and (d) strategies for immunomodulation, counteracting resistance and mitigating adverse events.

This year's Symposium will feature interactive sessions at the end of each day, where oncologists and researchers will provide their insights on the above mentioned topics in an effort to better understand tumor evolution and designing improved treatment strategies.

The 9th Symposium "Advances in Cancer Immunology and Immunotherapy" welcome the participation of scientists, clinicians, students and health care professionals interested in the field of Cancer Immunology and Immunotherapy.

We would like to thank you for your participation!

Warm regards,

The Organizing Committee

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MD, PhD, Professor of Medical Oncology, School of Medicine, University of Thessaly, General University Hospital of Larissa, Greece

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Scientific Director, Cancer Immunology and Immunotherapy Center "St. Savvas" Cancer Hospital, Athens, Greece

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Organizing Committee

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Constantin N. Baxevanis (GR)

Jörg Wischhusen (DE)

Sophia Karagiannis (GR)

9th Symposium on Advances in Cancer Immunology and Immunotherapy

Thursday, November 30th, 2023

08.50-09.00 **Opening: Organizing Committee**

09.00-10.10 **Session 1: Antitumor Immunity and Immunomodulation in the tumor microenvironment (part I)**
Chairs: **C.N. Baxevanis (GR), J. Wischhusen (DE)**

09.00-09.20	Immunosuppression in tumor Microenvironment and its targeting	**V. Umansky (DE)**
09.20-09.40	Insights into colorectal cancer immunity revealed by multidimensional spatial approaches	**N. de Miranda (NL)**
09.40-10.00	Interferon-induced lysosomal membrane permeabilization causes cDC1-deserts in tumors	**M. Tsoumakidou (GR)**
10.00-10.10	Discussion	

10.10-10.40 **Coffee Break**

10.40-12.15 **Session 2: Antitumor Immunity and Immunomodulation in the tumor microenvironment (part II)**
Chairs: **J. Wischhusen (DE), V. Georgoulias (GR)**

10.40-11.00	Pre-existing immunity in non-small cell lung cancer	**A. Xagara (GR)**
11.00-11.20	The cold immune environment in pancreatic cancer	**V. Bronte (IT)**
11.20-11.40	Microbiota-gut-brain axis in glioblastoma development	**O. Martin (FR)**
11.40-12.00	Immuno-microenvironment in infection and cancer	**T. Frisan (SE)**
12.00-12.15	Discussion	

12.15-12.45 **Keynote lecture**
Chair: **A. Kotsakis (GR), O.E. Tsitsilonis (GR)**

Tumor-derived exosomes in plasma of cancer patients induce stress and death in immune cells: danger from within **T. Whiteside (US)**

12.45-14.00 **Light Lunch**

14.00-15.00 **Short presentations**
Chairs: **I. Pateras (GR), O. Martin (FR)**

14.00-14.15	T-CELL RECEPTOR AND IMMUNE-RESPONSE GENE STUDIES IN TUMOR MICROENVI-RONMENT AND PERIPHERAL BLOOD IN HEAD AND NECK CANCER PATIENTS	**P. Batsaki**
14.15-14.30	THE TCR Vβ REPERTOIRE COMPOSITION AS A PREDICTIVE BIOMARKER OF IMMUNOTHERAPY EFFICIENCY IN NON-SMALL CELL LUNG CANCER PATIENTS	**P. Batsaki**
14.30-14.45	KMT2C IS A TUMOR SUPPRESSOR IN BLADDER CANCER AND ITS LOSS LEADS TO FAVORABLE RESPONSE TO IMMUNOTHERAPY	**F. E. Koukouzeli**
14.45-15.00	REAL-WORLD DATA ON IMMUNOTHERAPY IN PATIENTS WITH SOFT TISSUE SARCOMA: A STUDY FROM THE HELLENIC GROUP OF SARCOMA AND RARE CANCERS	**E. Georgaki**

15.00-16.10 **Session 3: Novel anticancer therapies (Part I)**
Chairs: **C.N. Baxevanis (GR), V. Umansky (DE)**

15.00-15.20	CAR- and TCR-based therapy in solid cancers	**E. M. Inderberg (NO)**
15.20-15.40	The power of novel allogeneic CAR-T cell to treat malignant disorders	**A. Madrigal (UK)**
15.40-16.00	Tolerogenic onco-fetal proteins as emerging targets for novel therapies	**J. Wischhusen (DE)**
16.00-16.10	Discussion	

16.10-16.30 **Coffee Break**

16.30-17.00 **Keynote lecture**
Chairs: **C.N. Baxevanis (GR), J. Wischhusen (DE)**

Peripheral blood biomarkers predicting clinical response to checkpoint blockade in melanoma — **G. Pawelec (DE)**

17.00-17.20 **Educational lecture**
Chairs: **A. Kotsakis (GR), G. Pawelec (DE)**

Immunotherapy in melanoma: past-present-future — **A. Laskarakis (GR)**

17.20-19.00 **Interactive session with the Speakers from sessions 1-3**

Friday, December 1st, 2023

09.00-10.10 **Session 4: Cancer Biomarkers (Part I)**
Chairs: **S. Karagiannis (UK), E. M. Inderberg (NO)**

09.00-09.20 The biomarker utility of TCR repertoire for clinical responses to immunotherapy in NSCLC — **M. Goulielmaki (GR)**
09.20-09.40 Dissecting the tumor microenvironment in early breast cancer: clinical implications and novel insights — **A. Matikas (SE)**
09.40-10.00 Immune-based biomarkers in solid tumors — **I. Pateras (GR)**
10.00-10.10 Discussion

10.10-10.40 **Coffee Break**

10.40-11.50 **Session 5: Novel anticancer therapies (part II)**
Chairs: **B. Selinger (DE), T. Frisan (SE)**

10.40-11.00 Therapeutic targeting of Treg cells in cancer — **P. Verginis (GR)**
11.00-11.20 Fine-tuning antigen presentation for cancer immunotherapy — **E. Stratikos (GR)**
11.20-11.40 Immunotherapy for cancer via reprogramming macrophages with IgE antibody — **S. Karagiannis (UK)**
11.40-11.50 Discussion

11.50-12.10 **Educational lecture** *sponsored by* AstraZeneca
Chairs: **A. Kotsakis (GR), V. Georgoulias (GR)**

Combining radiotherapy and immunotherapy — **M. Koukourakis (GR)**

12.10-13.30 **Light Lunch**

13.30-15.00 **Short Presentations**
Chairs: **S. Karagiannis (UK), A. Chatzigeorgiou (GR)**

13.30-13.45 CHARACTERIZATION OF A B-LIKE PHENOTYPE CELL FOUND IN COLON ADE-NOCARCINOMAS — **A. Noa-Bolaño**
13.45-14.00 THE IMMUNE STATUS OF COLORECTAL CANCER PATIENTS REVEALS A DEEP IMPAIRMENT IN RELEVANT CELLULAR EFFECTORS OF THE IMMUNE SYSTEM — **U. Rahner**
14.00-14.15 ASSESSING THE BEHAVIOUR OF THE GENOTOXIN-PRODUCING SALMONELLA ENTERICA IN PRO-CARCINOGENIC MOUSE MODELS — **M.L. Chiloeches**
14.15-14.30 MICROENVIRONMENTAL CONDITIONS AND HLA-CLASS-I EXPRESSION OF BREAST CANCER — **E. Xanthopoulou**
14.30-14.45 NEOADJUVANT CHEMORADIOTHERAPY OF RECTAL CANCER AND HLA-CLASS-I-RELATED MOLECULES EXPRESSION — **I.M. Koukourakis**
14.45-15.00 GDF-15 - A CYTOKINE AND ITS IMMUNOMODULATORY POTENTIAL — **B. Haack**

15.00-16.10 **Session 6: Cancer Biomarkers (Part II)**
Chairs: **C.N. Baxevanis (GR), S. Karagiannis (UK)**

15.00-15.20 T cell therapy in lung cancer **T. Tseveleki (GR)**
15.20-15.40 High throughput proteomics for the detection of cancer biomarkers **H.S. Ryu (US)**
15.40-16.00 ImmunoSpot assays permit B cell affinity measurements **P. Lehmann (US)**
16.00-16.10 Discussion

16.10-16.40 **Coffee Break**

16.40-17.10 **Keynote lecture**
Chairs: **J. Wischhusen (DE), A. Psyrri (GR)**

Oncogenes and tumor suppressor genes as regulators for immune surveillance **B. Seliger (DE)**

17.10-17.30 **Educational lecture**
Chairs: **A. Kotsakis (GR), T. Whiteside (US)**

Head and neck cancer: past, present, future **A. Psyrri (GR)**

17.30-19.30 **Interactive session with the Speakers from sessions 4-6**

Saturday, December 2nd, 2023

09.00-10.35 **Session 7: New topics in cancer immunology and immunotherapy**
Chairs: **S. Karagiannis (UK), N. de Miranda (NL)**

09.00-09.20 Onco-fibrotherapy in pancreatic cancer: Can the Cancer wound heal? **D. Öhlund (SE)**
09.20-09.40 Bio-informatic analysis in cancer immunology and immunotherapy **S. Tsoka (UK)**
09.40-10.00 Progress in the development of a clinically viable MYC inhibitor **L. Soucek (ES)**
10.00-10.20 Epigenetic regulation in cancer **A. Klinakis (GR)**
10.20-10.35 Discussion

10.35-11.00 **Coffee Break**

11.00-12.10 **Session 8: Immunotherapies alone in combination with other therapies (Part I)**
Chairs: **A. Kotsakis (GR), A. Klinakis (GR)**

11.00-11.20 Emerging standards in the perio-operative treatment in NSCLC **E. Fergadis (GR)**
11.20-11.40 State of the art of immunotherapy in the first line setting in NSCLC **D. Papadatos-Pastos (UK)**
11.40-12.00 The role of immunotherapy in gastric and gastroesophangeal cancer **A. Strimpakos (GR)**
12.00-12.10 Discussion

12.10-13.30 **Lunch**

13.30-15.00 **Short Presentations**
Chairs: **J. Wischhusen (DE), A. Xagara (GR)**

13.30-13.45 PROGNOSTIC SIGNIFICANCE OF SHP2 EXPRESSION IN NON-SMALL LUNG CANCER PATIENTS **E.A. Karatrasoglou**
13.45-14.00 CONCOMITANT CAUSES OF ANAEMIA IN A PATIENT WITH LUNG ADENOCARCINOMA UNDER anti-PD-1 IMMUNOTHERAPY **A. Patsouras**
14.00-14.15 DISTINCT PROFILE OF PROLIFERATING CD8+/TCF1+ T CELLS AMONG DIFFERENT BREAST CANCER MOLECULAR SUBTYPES **K. Ntostoglou**
14.15-14.30 Fc ENGINEERED MONOCLONAL ANTIBODIES FOR TRIPLE NEGATIVE BREAST CANCER THERAPY **A. Chenoweth**
14.30-14.45 EFFECT OF THE MEK INHIBITOR COBIMETINIB ON BRAF V600E MUTATED THYROID CARCINOMA **M. Udinotti**
14.45-15.00 OVERALL DISCUSSION

15.00-16.00 **Session 9: Immunotherapies alone in combination with other therapies (Part II)**
Chairs: **J. Wischhusen (DE), F.I. Dimitrakopoulos (GR)**

15.00-15.20 Immunotherapy in breast cancer or **K. Trifonidis (US)**
15.20-15.40 Immunotherapy in urothelial carcinomas **P. Tsantoulis (CH)**
15.40-16.00 Discussion

16.00-16.30 **Coffee Break**

9th Symposium on Advances in Cancer Immunology and Immunotherapy

16.30-17.00 **Keynote lecture**
Chairs: **A. Kotsakis (GR), V. Georgoulias (GR)**

Persistent Mutation Burden Drives Sustained Anti-tumor Immune Response **V. Anagnostou (US)**

17.00-17.10 **Abstracts Awards**
Organizing Committee

17.10-19.10 **Interactive session with the Speakers from sessions 7-9**

19.10-19.25 **Concluding remarks**
J. Wischhusen (DE), S. Karagiannis (UK)

General info

Organized by

Hellenic Society of Immunooncology
5, G. Theologou Str., P.C. 11474, Athens - Greece

Date
30/11 - 02/12, 2023

Venue
Royal Olympic Hotel, Athens

Hybrid Event
The conference will take place at the Royal Olympic Hotel, Athens, Greece.
You can watch the Conference online through the Link https://avolution.nowlive.events/1671immuno/web/ and ask written questions throughout the presentations.

Official language
The official language of the Meeting is English.

Accreditation / Certificate of Attendance
The Symposium has be accredited by the European Accreditation Council for Continuing Medical Education (EACCME®) with **19** CME credits (ECMEC®s).
The certificate of attendance will be given to the participants at the end of the event.
Based on the latest circular of the National Drug Organization the Event is required to use an attendance tracking system. By the end of the event, a certificate will be given to those who have attended at least 60% of the total hours of the scientific Program.

Symposium Secretariat

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9th Symposium on Advances in Cancer Immunology and Immunotherapy

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Abstracts

T-CELL RECEPTOR AND IMMUNE-RESPONSE GENE STUDIES IN TUMOR MICROENVI-RONMENT AND PERIPHERAL BLOOD IN HEAD AND NECK CANCER PATIENTS

Panagiota Batsaki[1], Maria Goulielmaki[1], Andriana Razou[2], Athanassios Sakellaridis[2], Niki Arnogiannaki[3], Angelos D. Gritzapis[1], Constantin N. Baxevanis[1], Sotirios P. Fortis[1]

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Background: Worldwide, head and neck cancer (H&N) accounts for approximately 900,000 new cases and over 400,000 deaths annually. An emerging tumor microenvironment (TME) is a complex and continuously evolving entity. T-cell receptor (TCR) repertoire and levels of expression of immune-related genes (IRGs), were widely introduced as important tools to reveal the status of the immune response intratumorally, as well as in the circulation. Furthermore, genetic alterations could be used as molecular targets for treatment, as well as biomarkers with prognostic utility. The aim of this study was to investigate the tumor mutational load, along with alterations in IRGs expression levels and in the TCR repertoire, in the peripheral blood and TME of H&N cancer patients.

Methods: DNA and RNA were isolated from peripheral blood and from tumor tissue (Formalin-fixed paraffin-embedded sample-FFPE) of 26 H&N cancer patients, followed by Next-Generation Sequencing (NGS). The Ion Oncomine™ TCR Beta-SR Assay was used in order to identify and measure the clonal expansion of T-cells. The IRG profiling was studied using a panel of 398 immune-related genes (On-comine™ Immune Response Research Assay). Alterations in the TCR repertoire and in IRG expression levels were evaluated in the peripheral blood and the TME. The genetic alterations in the TME were detected using the RingCap® Human Pan-Cancer Drive Gene Mutations Kit.

Results: We found 80 IRGs that were down - and 72 IRGs that were up - regulated in the TME compared to matched peripheral blood samples. In a similar fashion, we identified alterations in the frequencies of TCR clonotypes between the circulating and infiltrating T-cells. We also detected hotspot variants in 38 common tumor driver genes, among which point mutations and small fragment insertion and deletion mutations in the TME.

Conclusions: Differences in TCR repertoire and in IRGs expression between TME and circulation could potentially act as dynamic biomarkers in H&N cancer. The mutations detected could be variants with clinical significance or potential drug targets. These findings need to be further confirmed in larger patients' cohorts.

Funding: This study was co-funded by the European Regional Development Fund and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call Research–Create–Innovate (project code: T2EDK-03266, project acronym and title: "BIOKAKETRA - Identification of genomic and transcriptomic prognostic biosignatures in head and neck cancer").

THE TCR Vβ REPERTOIRE COMPOSITION AS A PREDICTIVE BIOMARKER OF IMMUNOTHERAPY EFFI-CIENCY IN NON-SMALL CELL LUNG CANCER PATIENTS

Panagiota Batsaki[1], Sotirios P. Fortis[1], Angelos D. Gritzapis[1], Anastasia Xagara[2], Athanasios Kotsakis[2,3], Constantin N. Baxevanis[1] and Maria Goulielmaki[1]

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Background: Immunotherapy is widely utilized for the treatment of solid tumors including non-small cell lung cancer (NSCLC). Considering that immune checkpoint inhibitors (ICIs) act through reinvigorating the endogenous antitumor T-cell immunity, facilitating cancer cell recognition and elimination by specific T-lymphocytes, it can be assumed that the TCR Vβ repertoire will change upon treatment with ICIs. The aim of this study is to investigate immunotherapy-induced changes in the peripheral TCR Vβ repertoire of NSCLC patients.

Methods: Thirty-six PD-L1(+), stage III NSCLC patients, eligible to receive durvalumab post radio-chemotherapy, have been recruited in this study (study group). Another twenty-one patients were assigned to receive either chemoradiotherapy or immunotherapy alone, or combined immuno-/chemioradiotherapy (control group). DNA was isolated from the peripheral blood of patients in the study group at baseline (before immunotherapy), and where available, at 1st evaluation (three months after immunotherapy initiation) and at the end of the treatment course or at disease progression. DNA was also extracted from the available tumor tissue samples of patients belonging in both groups. Next-generation-sequencing was performed and the Ion Oncomine™ TCR Beta-SR Assay was used for the identification and quantification of T-cell clonal expansion.

Results: Our findings demonstrate alterations in the peripheral TCR Vβ repertoire post immunotherapy. Based on clonotype counts three months after treatment initiation, two groups of patients were identified, those with i) decreased or ii) increased number of TCR clonotypes. Significant changes were observed regarding the frequencies of distinct TCR clonotypes at the different time points of the study. New TCR clonotypes with increased frequencies emerged in fourteen patients, while eight patients had clonotypes that disappeared at 1st evaluation. Significant differences were also identified between the patients' periphery and tumor.

Conclusions: Significant alterations in the TCR Vβ composition were identified post durvalumab treatment. The emergence of new TCR clonotypes and the extinction of others were also important findings. Such alterations in the TCR Vβ repertoire may hold potential as dynamic biomarkers of response to treatment.

Funding: This research has been co-financed by the European Regional Development Fund of the European Union and Greek National Funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T2EDK-02218, project acronym and title: "B-PREIMMUN, Immune and Molecular circulating biomarkers for the selection of cancer patients for immunotherapy").

KMT2C IS A TUMOR SUPPRESSOR IN BLADDER CANCER AND ITS LOSS LEADS TO FAVORABLE RESPONSE TO IMMUNOTHERAPY

Fotini E. Koukouzeli[1], Yiannis Ntounias[1], Konstantina I. Georgakopoulou[1], Theodoros Loupis[1], Apostolos Klinakis[1]

[1] Biomedical Research Foundation of the Academy of Athens, Athens, Greece

I. Background: Bladder cancer stands out among solid malignancies due to the high percentage of tumors with inactivating mutations in histone-modifying proteins. Histone 3 lysine 4 (H3K4) methyltransferases are commonly mutated in bladder cancer. Among those, the H3K4 methyltransferase KMT2C/MLL3 is mutated in more than 20% of cases. We and others have generated data implicating KMT2C/MLL3 in DNA repair and therapeutic response. Defects in DNA repair, genomic instability and tumor mutation burden (TMB) are believed to contribute to tumor immunogenicity and thus, favorable response to immune checkpoint inhibitors (ICIs). The aim of this study is how epigenetic deregulation, and specifically KMT2C/MLL3 loss contributes to these phenomena.

II. Methods: Here, we employ published TCGA cohorts of primary and metastatic bladder cancer patients to study the consequences of KMT2C/MLL3 loss in the tumor genetic profile. Moreover, we developed a novel mouse model of tissue-specific inactivation of KMT2C/MLL3, which allowed us to study *in vitro* and *in vivo* the role of KMT2C/MLL3 in tumor initiation/progression and response to ICIs.

III. Results: Loss-of-function truncating mutations in KMT2C/MLL3 in human bladder cancer cohorts are associated with high TMB, presumably as a result of increased insertions/deletions (Indels) frequency. Functional experiments in mouse models indicated that KMT2C/MLL3 is a *bona fide* tumor suppressor in bladder cancer. Tissue-specific knockout of KMT2C/MLL3 activity leads to increased clonogenic potential in an *in vitro* organoid assay. Moreover, in a mouse model of chemical

carcinogenesis, KMT2C/MLL3 knockout leads to reduced tumor latency and mouse survival. Exome-sequencing in mouse tumors indicated a rise in Indel frequency in KMT2C/MLL3 knockout mice. In a preclinical study with a mouse PD-1 blocking antibody, we showed that while control animals under treatment gain an average of 26 days survival (54 days vs. 28 days in the vehicle control cohort), KMT2C/MLL3 knockout counterparts show a more robust response, gaining 70 days (92.5 vs. 22 days).

IV. Conclusions: This study highlights the functional implications of KMT2C/MLL3 loss in tumor initiation and immunotherapy response in bladder cancer.

REAL-WORLD DATA ON IMMUNOTHERAPY IN PATIENTS WITH SOFT TISSUE SARCOMA: A STUDY FROM THE HELLENIC GROUP OF SARCOMA AND RARE CANCERS

Eleni Georgaki, Anastasios Kyriazoglou, Maria Kirkasiadou, Anna Boulouta, Anna Koumarianou, Eleftherios Delivorias, Ioannis Boukovinas, Vasilis Georgoulias, Stefania Kokkali

Oncology Unit, 2nd Department of Medicine, National and Kapodistrian University of Athens, Medical School, Hippocratio General Hospital of Athens; V.Sofias 114, 11527 Athens, Greece

Background: Following the advancement of immunotherapy (IO) in other cancer types, there is heightened interest in exploring its role in soft tissue sarcoma (STS) treatment. Immune checkpoint inhibitors (ICIs) have been investigated in small phase 2 clinical trials. We aimed to assess the real-world clinical outcomes and safety of ICIs in Greek STS patients.

Methods: Clinical data of STS patients treated with off-label ICIs (either as monotherapy or combinations) from 2020 to 2023 at seven centers within the Hellenic Group of Sarcoma and Rare Cancers were retrospectively analyzed. IO biomarkers were also registered. Primary end-point was progression-free survival (PFS). Secondary end-point was overall survival (OS). Adverse events were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Results: 19 patients with metastatic STS were identified. Surgery of the primary tumor was performed in 16 and (neo) adjuvant chemotherapy in 10 patients. Patients had received 0-6 (median 2) prior treatment lines for advanced disease. Biomarkers related to IO were available for 10 patients: PDL1 expression in immune cells was positive (low) in 2/7 patients, tumor mutational burden (TMB) was high in 2/9 patients and microsatellite instability (MSI) was high in 1/10 patients. Five patients exhibited partial response as best response to IO, leading to a response rate of 26.3%. All responders received ICIs as 1st or 2nd-line treatment and included one patient with positive PDL1 expression, two with high TMB and the patient with MSI high. Four patients exhibited stable disease, with following histotypes: synovial sarcoma, malignant granular cell tumor, epithelioid sarcoma and clear cell sarcoma-like gastrointestinal tumour (CCSLGT). Median PFS was 3.5 months (0-24.4) and median OS 4.6 months (1.7-34). Three patients experienced sustained response to ICI with PFS >6 months: the patients with ASPS, uterine LMS and CCSLGT. Grade 1-2 adverse events (colitis, anorexia, fatigue, skin toxicity) were recorded.

Conclusions: With the limitation of a small sample size and a retrospective study design, this analysis shows that ICIs benefit a subset of STS patients.

CHARACTERIZATION OF A B-LIKE PHENOTYPE CELL FOUND IN COLON ADE-NOCARCINOMAS

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Background: The distribution of immune cells in colorectal cancers (CRCs) is scattered throughout the tumor and stromal regions and even the surrounding tissues. However, the com-position and function of infiltrated immune cells, which make up a tumor immune microenvi-ronment (TIME), is complex and not completely understood. Nevertheless, TIME is strongly associated with tumor development, progression, and outcomes. It is currently unclear whether the immune cells observed

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in crosstalk with tumors interfere with the phenotypic plasticity of cancer cells and contribute to the clonal evolution, tolerance, and recurrence of malignant tu-mors after therapeutic interventions. In this study, we focused on a distinctive cell type with B cell-like phenotype, commonly found in colon adenocarcinomas, which we have named "cala-mari" due to their particular dynamic movements.

Methods: We aimed to investigate the immune characteristics of "calamari" cells, their cell differentiation and stemness features, and their strong neuro-immune signature. We employed qPCR to analyze at transcriptional level the expression of markers for stemness and neuro-phenotype, corroborating the expression of such markers at protein level using immunotech-niques like FACS, western blots, and ICC. In addition, their morphology was addressed using TEM, SEM and bright field videography. Wide transcriptome analyses were performed using NGS.

Results: We found that "calamari" cells interact actively with cancer-associated fibroblasts (CAF). Analysis of calamari cells revealed in part their immune nature, as judged by the ex-pression of CD45, CD19, MHCII, and immunoglobulin production. However, CD19 expression was heterogeneous among sub-clones, indicating that not all cells were typical B cells. Interest-ingly, they displayed a signature congruent with intestinal M cells, as revealed by their reactivi-ty to UEA-1, Gp2, and TNFaIP2. Notably, these cells also expressed several classical neuronal markers as well as an overexpression of several transcriptional factors typically found in stem-ness context. Transcriptome analysis indicated a prior infection history of these cells, notably from herpesviruses Epstein-Barr and Kaposi Sarcoma viruses, as well as by the retrovirus HTLV-I. However, only EBV genes were found to be actively expressed after validating the NGS results using TaqMan-based qPCR.

Conclusions: Our study sheds light on the unique "calamari" cell type found in colon adenocar-cinomas. These cells exhibit a complex immune profile, including signs of prior infections. Their distinctive neuro-immune characteristics open avenues for further exploration into their cellular origin as well as their role in the tumor microenvironment. Whether they represent a neuro-immune cell type formed through the convergence of 2-3 different cell types with cell-specific epigenetics must be further studied. Understanding these cells may offer insights into cancer progression and therapeutic strategies.

THE IMMUNE STATUS OF COLORECTAL CANCER PATIENTS REVEALS A DEEP IMPAIRMENT IN RELEVANT CELLULAR EFFECTORS OF THE IMMUNE SYSTEM

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Background: The immune status (IS) principally refers to the regular cell composition of the immune system as measured in peripheral blood, enabling a detailed distribution of immune cells as well as the grade of activation of the patient's immune system. Thus, the IS analysis in cancer patients could reveal important clues on the interaction of the immune system with tumors. Unfortunately, there is scarce work on the numerical and the physiological steady state characterization of the peripheral immune cell effectors and their dynamics in oncological patients.

Methods: We monitored the IS in colorectal carcinoma (CRC), therapy-naïve patients, using flow cytometry, which allows a very informative phenotyping analysis of immune cells. For this purpose, we compared the IS of 82 CRC patients (median age 72) with two control groups [young (median age 24) and old (median age 83) individuals], which were not previously diagnosed with any malignancy.

Results: We found that CRC patients develop granulocytosis and lymphopenia that was principally observed in the CD4 subset, which in addition, showed high levels of the activation marker HLA-DR. Contrarily, the percentages of CD8 and CD19 populations were unaltered. As expected, the innate immune effector NK cells were at the increased population level, but as judged by the low expression of CD158, rather in a non-functional status. The analysis of different immune checkpoints in $CD4^+$ and $CD8^+$ cells from patients with CRCs revealed a down-regulation of CTLA-4. Moreover, the expression of CXCR4 on $CD4^+$ and $CD8^+$ was found to be high.

Conclusions: The general granulocytosis observed in CRC patients might be associated with lymphopenia, as compensation. However, the significant reduction of the CD4 population and its activation, as a possible cause of their decay, might have an impact on the proper responsiveness of the immune system. Moreover, despite the numerical increase in the NK cell population, their low CD158 expression indicates functional impairment. In addition, the high expression of CXCR4 on CD4 and CD8 lymphocytes provides important clues to the migration patterns of these populations towards tumors. The observed downregulation of CTLA-4 indicates that the use of immunological interventions in this tumor entity targeting this checkpoint may be limited. Taken all together, the data indicate an impaired immune system in CRC patients. Yet, this research should be extended to additional aspects concerning the physiology of immune cells and correlate the information with the presence and functionality of tumor-infiltrated leucocytes.

ASSESSING THE BEHAVIOUR OF THE GENOTOXIN-PRODUCING SALMONELLA ENTERICA IN PRO-CARCINOGENIC MOUSE MODELS

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Some Salmonella enterica serovars as Typhi and Paratyphi secrete a toxin known as typhoid toxin (TT), which induces DNA single - and double - strand breaks in host cells via their cdtB subunit, an homologue to the mammalian DNase I. Induction of DNA damage activates DNA repair mechanisms. However, if the DNA damage is beyond repair, most cells undergo a permanent, proinflammatory cell cycle arrest known as se-nescence. Besides, some damaged cells with procarcinogenic features overcome the tumorigenic barrier and proliferate. The induction of genomic instability by DNA-damaging toxins has been widely studied in vitro and in vivo in immunodeficient models. However, little is known about the pro-carcinogenic role of the TT in in vivo immunocompetent models. Here, we established two chemically-induced cancer models by using the pro-carcinogenic agent azoxymethane (AOM) alone or in combination with the colitisinducing compound dextran-sodium sulphate (AOM/DSS) to study the contribution of infection by TT-producing Salmonella for cancer development. We detected a higher bacterial colonisation of the TT-producing Salmonella in colon, compared to the isogenic control strain in the AOM model. The increased colonisation correlated with the activation of the DNA Damage Response, as assessed by staining of the surrogate marker γH2AX, mainly in cells conforming the crypts. Stromal cells underwent mostly oxidative damage, as tested by staining with the marker 8- oxoguanine. We detected a bacterial-dependent sustained inflammation in both models, charac-terised by the formation of lymphocytic aggregates. Only in few mice infected with the genotoxigenic Salmo-nella development of neoplasia was observed in the AOM/DSS model. The number of these aggregates was bacteria-dependent, but toxin-independent. Interestingly, and despite the strong inflammatory microenvi-ronment upon infection, we did not observe induction of senescence in none of the conditions analysed. Our results highlight the complexity of the tissue microenvironment and point towards a fine-tuned crosstalk between the immune system and the DNA Damage Repair system to hijack tissue homeostasis during infec-tion

MICROENVIRONMENTAL CONDITIONS AND HLA-CLASS-I EXPRESSION OF BREAST CANCER

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Background: The use of immunotherapy in the treatment of breast cancer presents a unique opportunity to investigate alterations in the cancer cell immuno-phenotype, which may be utilized to enhance the effectiveness of immunotherapy interventions and ultimately improve patient outcomes.

Methods: For the current experimental procedure, human breast cancer cell lines MCF7 and T47D were used. The cells were exposed to hypoxic conditions in a Hypoxia Chamber (mixed gas 5% CO2 /1% O2 /74% N2) for 48 h and in acidic in

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D7777 medium with pH 6.2 (balanced in a 5% CO2 incubator) without sodium bicarbonate, for 24 h. Additionally, cells were co-cultured for 48 h with healthy donor's PBMCs. Moreover, cells were exposed to 25ng/ml IFNγ for 48 h or a combination of hypoxic conditions with 25ng/ml IFNγ. Western blot Analysis and Flow Cytometry was performed for the evaluation of the levels of HLA Class I ABC protein.

Results: In Western blot analysis, T47D had high levels of HLA-class-I, while low levels were recorded in the MCF7 one. Incubation in hypoxic conditions suppressed the expression of HLA-class-I in both cell lines, while acidity had no effect. In Flow-Cytometry experiments, MCF7 cell line incubation with hypoxic conditions resulted in a significant decrease of the percentage of cancer cells expressing HLA-class-I in the gated area. Incubation with PBMCs significantly increased the expression of HLA-class-I positive cancer cells. A strong increase was detected after incubation with IFNγ. This effect of IFNγ was clearly compromised under hypoxic conditions. Similar results were obtained with the T47D cell line. Hypoxia decreased the HLA-expressing cancer cells, while co-culturing with healthy donor's PBMCs and IFNγ increased the % of HLA-class-I cancer cells.

Conclusions: Tumor microenvironmental conditions, like hypoxia and IFNγ secreted by immune cells, play an important role in HLA-class-I expression regulation and, eventually, in immune surveillance in breast cancer cell lines revealing a potential role on future strategies of immunotherapy.

NEOADJUVANT CHEMORADIOTHERAPY OF RECTAL CANCER AND HLA-CLASS-I-RELATED MOLECULES EXPRESSION

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Background: The HLA-class-I molecules are essential for the presentation of non-self-antigens by cancer cells to dendritic and cytotoxic T-cells. Loss of HLA-class-I molecule expression is a process through which cancers cells attempt to evade immune surveillance. The radio-vaccination effects of radiotherapy (RT) can be utilized as a means to overcome this immunotherapy-resistance mechanism.

Methods: In the current study we investigated the effects of RT on the expression of HLA-class-I-related molecules in 21 rectal cancer patients treated with neoadjuvant chemo-RT of 50.4Gy and concurrent capecitabine. Immunohistochemistry analysis was performed on 3 μm-thick formalin-fixed, paraffin-embedded (FFPE) tissue sections from matched pre-treatment biopsy and post-RT surgical material, placed on positively charged slides. The ab70328 mouse monoclonal antibody (abcam, UK) recognizing the heavy chains of human HLA-class-I A, B, and C (concentration 1/200, 60min incubation), the ab21899 mouse monoclonal antibody (abcam, UK) recognizing the human β2-microglobulin (concentration 1/50, overnight incubation) and the ab83817 rabbit polyclonal antiserum (abcam, UK) recognizing the human TAP1 protein (concentration 1/50, overnight incubation) were utilized. Furthermore, the effect of 4 and 10 Gy irradiation on HLA-class-I expression in colorectal adenocarcinoma cell lines HT-29 and Caco-2 was examined.

Results: HLA-class-I, β2-microglobulin and TAP1 were poorly expressed in 47.6%, 47.6% and 38% of cases, respectively, before treatment with RT, while only 2 cases (9%) displayed extensive expression of all 3 proteins. Post-RT, a significant upregulation of the HLA-related molecules was observed, and 15 out of 21 cases (71%) demonstrated extensive expression

of all 3 proteins (p<0.0001). Finally, HLA-related molecules were induced in both irradiated cancer cell lines as shown in Western Blot, RT-PCR and Flow-Cytometry analysis.

Conclusions: HLA-class-I related molecules are significantly upregulated after neoadjuvant RT of patients with locally advanced rectal cancer. This is also confirmed in *in vitro* experiments. Further investigation is required to exploit this important radio-vaccination effect aiming to enhance the efficacy of immunotherapy in rectal cancer

GDF-15 - A CYTOKINE AND ITS IMMUNOMODULATORY POTENTIAL

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Growth/differentiation factor-15 (GDF-15) is a distant member of the transforming growth factor-β superfamily. Under normal conditions, GDF-15 shows very low constitu-tive expression in most somatic tissues. However, its expression is induced under stress conditions as well as during pregnancy in the placenta. Moreover, GDF-15 is a bi-omarker for disease prognosis in cancer. One well-documented function, induction of anorexia, is mediated via the brainstem-restricted GDF-15 receptor GFRAL (glial cell-derived neurotrophic factor [GDNF] family receptor α-like). Still various other roles, such as during pregnancy and tumor progression, cannot be explained via the known mecha-nism due to the absence of GFRAL on immune cells. Mechanistically, *in vitro* data show that GDF-15 impairs activation of LFA-1/β2-integrin on T cells, which is critically required for interaction with ICAM expressed by endothelial cells and antigen presenting cells. Thereby, GDF-15 does not only destabilize interactions with DCs during T cell priming, but also their extravasation to tissues and infiltration into tumors. These findings were supported by *in vivo* data from the MC38 colon cancer and the orthotopic Panc02 pan-creatic tumor model. Besides affecting immune cell trafficking, GDF-15 can also skew macrophages towards a more anti-inflammatory, tumor-promoting phenotype. GDF-15 thus induces immune tolerance on many different levels, which supports its targeting for cancer immunotherapy.

PROGNOSTIC SIGNIFICANCE OF SHP2 EXPRESSION IN NON-SMALL LUNG CANCER PATIENTS

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Background: Lung cancer remains the leading cause of cancer-related death across the world. Eighty-five percent of lung cancers are of the non–small cell type (NSCLC). Use of immune checkpoint inhibitors alone or in combination with chemotherapy, has become a gold standard for these patients. SHP-2 is a nonreceptor, ubiquitous protein tyrosine phosphatase required for the growth of KRAS mutant and ALK-rearranged NSCLC. In T cells, SHP-2 is an intracellular molecule activated downstream of the PD-1 signaling pathway that suppresses T-cell activation and thereby antitumor immunity.

Methods: We examined the association between SHP2 expression and PD-L1 expression along with driver gene mutations in 259 Greek NSCLC patients. We also examined the possible correlation between SHP2 expression and clinical response to immunotherapy. SHP2 expression was estimated by immunohistochemistry (D50F2 Rabbit mAb) with any H-score ≥ 150 considered as positive. PD-L1 immunoexpression was defined using 22C3 PharmDx DAKO (mAb). Driver gene mutations were detected by Next Generation Sequencing (NGS). Data analysis was performed using SPSS v29.0.

Results: SHP2 positive expression was detected in 61 cases (23.5%). KRAS mutated patients with positive SHP2 expression showed a statistically significant benefit both in overall survival (OS) (p 0.033) and progression free survival (PFS) (p 0.003) compared to KRAS mutated SHP2 non-expressors irrespective of treatment. SHP2 expression is a strong prognostic factor as it was also independently correlated with PFS benefit (p 0.0024). Upon stratification according to treatment, SHP2 positive patients who received immunotherapy as first line therapy showed a statistically significant benefit in PFS compared with non expressors receiving the same treatment (p 0.0076).

Conclusions: Positive SHP2 expression appears to offer PFS benefit regardless of treatment option while this benefit

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is expanded and enhanced in patient group receiving immunotherapy. Of great interest is the fact that KRAS mutated/SHP2 expressors showed OS and PFS benefit compared to KRAS mutated/SHP2 non-expressors. KRAS mutations' poor prognostic effect is well documented. SHP2 expression may have a protective role in this patients' group while may be an effective indicator of response to immunotherapy. Additional prospective studies are warranted to validate this finding.

CONCOMITANT CAUSES OF ANAEMIA IN A PATIENT WITH LUNG ADENOCARCINOMA UNDER anti-PD-1 IMMUNOTHERAPY

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Background: Immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 are associated with autoimmune adverse events (irAEs) that can affect multiple systems including the hematopoietic. The most common hematologic AEs from immunotherapy (Haem-irAEs) are neutropenia, thrombocyto-penia, hemolytic and aplastic anemia.

Case presentation: We report a case of autoimmune haemolytic anaemia (AIHA) and pure red cell aplasia (PRCA) in a patient with lung adenocarcinoma treated with Pembrolizumab.

A 59yo woman presented to our clinic due to dry cough. Chest CT scan revealed a nodule (1.3 cm) in the right lower lobe and mediastinal and upper supraclavicular lymphadenopathy. Ebus-TBNA bronchoscopy was performed and the diagnosis was a stage IIIb lung adenocarcinoma (T1bN3M0) without driver mutations and high PD-L1 expression (TPS 56%). Pembrolizumab was started (200mg q21d) with significant clinical and imaging improvement.

13 months later, she complained about weakness and haematological parameters revealed a fall oh hemoglobulin (Hb) from 15 to 11g/dl, in a period of 21 days. She underwent upper endosco-py that was negative. Both erythropoietin and iron transfusion were administered but the anemia persisted. After 2 weeks, she was admitted to the hospital due to severe weakness and a value of Hb about 7g/dl. Laboratory examination revealed elevated serum lactate dehydrogenase level (1,600 IU/L), positive direct Coombs test, decreased haptoglobin level (<20 mg/dL) and posi-tive hemoglobinuria, which confirmed the presence of AIHA. However, reticulocyte count (re-ticulocyte 0.08%, corrected reticulocyte count 0.04%, reticulocyte production index 0.02%) was decreased, suggesting that additional causes of anemia other than AIHA may be present. Hence, bone marrow examination was conducted, which revealed normocellular marrow (cellularity 35%) and active granulopoiesis with markedly decreased erythroid cells (myeloid: erythroid=24.9:1). Specifically, a CD8+ T cell-mediated destruction of the red cell precursors was mediated through CD8+ T cells. Based on the results of bone marrow examination, PRCA was diagnosed for the patient.

It was decided to withhold immunotherapy and start intravenous corticosteroids. After 3 months of treatment with methylprednisolone 24mg/day, the Hb had stabilized at 15mg/dl.

Conclusions: The incidence of Haem-irAES in treatment with ICIs targeting PD-1/PD-L1 is about 4%. The average time of their appearance is 10 weeks, while they can appear at any time of the treat-ment. They are potentially life-threatening conditions, with both AHIA and PRCA being seri-ous. In the present case, the onset of anemia was delayed while there was an immediate re-sponse to corticosteroid treatment.

DISTINCT PROFILE OF PROLIFERATING CD8+/TCF1+ T CELLS AMONG DIFFERENT BREAST CANCER MOLECULAR SUBTYPES

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I. Background: Proliferating CD8+ T cells represent a state of activated T cells during the effector phase of the immune response that potentiates their effector function. T cell exhaustion is linked with the expression of the transcription factor T cell factor 1 (TCF1, also known as *TCF7* by the gene encoded). TCF1 is expressed by progenitor T cells, while its expression is decreased in terminally exhausted T cells.

II. Methods: The aim of the current study is to decipher the expression of proliferating CD8+/TCF1+ T cells employing Breast Tissue Microarrays (797 cases) with different molecular subtypes. For this propose, we employed Double Immunohistochemistry to investigate the co-expression of CD8 and Ki-67, the number of positive TCF1 cells in the stroma and the percentage (%) of positive cancer cells and the frequency of CD8-TCF1 in all molecular subtypes of Breast Cancer.

III. Results

- CD8+ T cell density was significantly increased in HER2+ and to a lesser extent in TNBC breast cancer patients.
- Assessment of CD8+Ki67+ / CD8+ ratio revealed a significant decrease in the fraction of prolifer-ating CD8+ T cells in TNBC and HER2+.
- Infiltration by CD8+TCF1+ cells was significantly elevated in HER2+ patients.
- Increased presence of TCF1+ stromal cells, in both HER2+ and TNBC.
- TCF1+ cancer cells were significantly reduced in TNBC.
- Increased CD8+, CD8+Ki67 and CD8+TCF1+ infiltration is associated with improved DFS in TNBC but not in luminal type A.
- Increased TCF1 in the stroma is a favorable prognostic factor for DFS in TNBC.

IV. Conclusions: This study demonstrates a unique immunophenotypic profile of proliferating CD8+ including CD8+TCF1+ T cells. These findings highlight the importance of providing insight in the func-tional state of TILs rather than merely assessing their number.

Fc ENGINEERED MONOCLONAL ANTIBODIES FOR TRIPLE NEGATIVE BREAST CANCER THERAPY

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Triple negative breast cancers (TNBCs), which represent 10-20% of all breast carcinomas, are highly aggressive and have a poor prognosis compared to the other breast cancer types. Monoclonal antibody (mAb) therapy has revolutionised the treatment of certain types of breast cancer, by targeting tumour cell surface molecules such as the human epidermal growth factor receptor 2 (HER2). However, the only mAb-based therapeutic treatment available for TNBC is an anti-PD-L1 checkpoint inhibitor in combination with chemotherapy, which benefits a small fraction of patients. Thus, the development of novel and more effective treatments are urgently needed.

In this study we report the design and engineering of novel mAbs targeting TNBC tumour surface markers. We identified the cell surface molecule folate receptor alpha (FRα) and engineered the anti-FRα mAbs as either an IgG1 or an Fc engineered IgG1 to improve the Fc-mediated effector functions. We also engineered anti-HER2 mAbs with the same Fc mutations to compare to the clinically available trastuzumab and the recently approved Fc-enhanced anti-HER2 mAb margetuximab.

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Our Fc engineered antibodies elicited stronger immune mediated effector functions as compared to wildtype IgG1, inducing greater direct cell killing by NK cell-mediated ADCC. In addition, our Fc engineered antibodies also increased production of pro-inflammatory cytokines by NK cells and macrophages, which in turn led to repro-gramming of macrophages towards a more pro-inflammatory and anti-tumoural state. In summary, we have developed novel Fc engineered mAbs targeting TNBC with greater potential treatment efficacy.

EFFECT OF THE MEK INHIBITOR COBIMETINIB ON BRAF V600E MUTATED THYROID CARCINOMA

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Background: Thyroid carcinoma (TC) is classified into subtypes with different molecular features and patients outcome. As BRAF *V600E* mutation is prevalent in TC subtypes with additional NRAS mutations in some TCs, BRAF inhibitor therapy is an option in advanced diseases. However, under therapy TC often display intrinsic or develop extrinsic resistance due to the induction of CRAF dimerization thereby subsequently activating the MAPK pathway.

Methods: TC cell lines were left untreated or treated with the MEK inhibitor Cobimetinib and the BRAF inhibitor Dabrafenib followed by the analysis of cell proliferation, migration and wound healing. The expression and/or function of epithelial-to-mesenchymal (EMT) markers and immune modulatory molecules was determined by qPCR, Western blot and/or flow cytometry, IL-8 secretion into the TC cell line supernatants by ELISA. Immunohistochemical stainings of 187 TC specimens were performed with antibodies directed against CD8, MHC class I, PD-L1, EMT markers and data were associated to clinical parameters.

Results: *In silico* analysis of public available datasets of 507 primary and 8 metastatic papillary TC demonstrated a high frequency of *BRAF* V600E mutations (62.4%), with 8.5% having *NRAS* mutations. Cobimetinib treatment of TC cell lines resulted in a stronger inhibition of the ERK1/2 phosphorylation than Dabrafenib, which was associated with a decreased cell proliferation and migration. These Cobimetinib-mediated changes of growth properties were accompanied by an increased phosphorylation of p53 and p27/Kip1 levels and reduced expression of inhibitors of apoptosis, like survivin, claspin and pro-caspase-3. Furthermore, Cobimetinib significantly decreased IL8 secretion, altered the EMT and enhanced the immunogenicity of TC cells by reducing the expression of immune checkpoints, but upregulating the expression of HLA class I antigens. Immunohistochemical analyses revealed higher PD-L1 and HLA class I expression levels, but low □-SMA and FN1 expression in undifferentiated compared to the differentiated papillary TC subtype, which was associated with an increased CD8 T cell frequency.

Conclusions: The in-depth analysis provided insights into (i) the immunological features of TC subtypes and (ii) the potential of MEK inhibition as a therapeutic approach for overcoming BRAF inhibitor resistances of TC due to the downregulation of tumorigenic properties and an increased immunogenicity of TC.

This work is supported by an intramural Roux grant of the Medical Faculty of the Martin Luther University Halle-Wittenberg, Halle, Germany

Notes

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